VERIFICATION OF TRANSLATION

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declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

2. That the attached document is a true and correct translation made by me to the best of my knowledge and belief of:-

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【Name of Document】 Drawings 1

【Name of Document】 Abstract 1

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【Proof】 Necessary

[Document Name] Specification

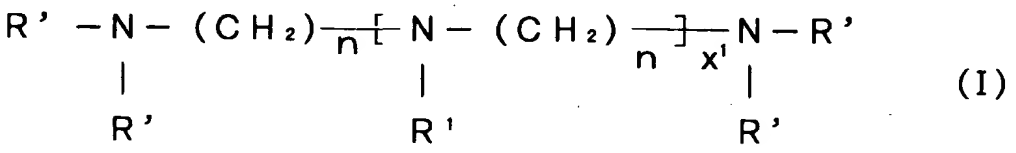
[Title of the Invention] Composition for transporting negatively charged substances

[Claims]

[Claim 1] A composition comprising a polyalkylenimine having two or more hydrophobic groups or its salt.

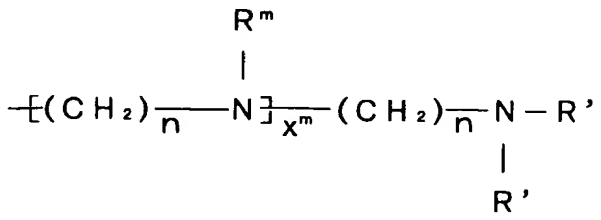
[Claim 2] The composition of Claim 1, wherein the hydrophobic group is a cholesterol residue, a saturated or unsaturated alkyl group, a saturated or unsaturated acyl group, or a phospholipid residue.

[Claim 3] The composition of Claim 1, wherein the polyalkylenimine having two or more hydrophobic groups is a compound represented by formula (I):

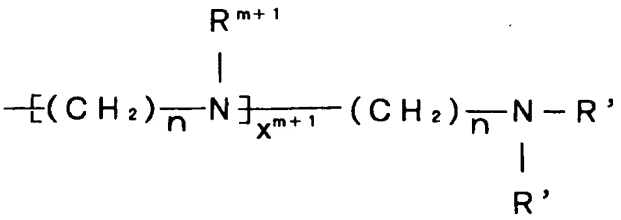


(I)

(wherein R' represents hydrogen, an alkyloxy group, or an alkoxy group; two R's binding to the same nitrogen atom can be identical or different; R^1 is hydrogen, a cholesterol residue, saturated or unsaturated alkyl group, saturated or unsaturated acyl group, a phospholipid residue, or below formula:



wherein R^m represents hydrogen, a cholesterol residue, a saturated or unsaturated alkyl group, a saturated or unsaturated acyl group, a phospholipid residue, or below formula:



wherein n, X^1, X^m, and X^{m+1} represent arbitrary natural numbers.)

[Claim 4] The composition of any one of Claims 1 to 3, wherein the polyalkylenimine is a polyethylenimine.

[Claim 5] The composition of any one of Claims 1 to 4, further comprising phospholipid.

[Claim 6] The composition of Claim 5, wherein the phospholipid is neutral or basic phospholipid.

[Claim 7] The composition of Claim 5, wherein the phospholipid comprises phosphatidylethanolamine, or phosphatidylcholine skeleton.

[Claim 8] The composition of Claim 7, wherein the phospholipid is dioleylphosphatidylethanolamine, or phosphatidylcholine.

[Claim 9] A complex comprising a physiologically active substance comprising a negative charge and a composition of any one of Claims 1 to 8.

[Claim 10] The complex of Claim 10, wherein the physiologically active substance comprising a negative charge is a nucleic acid or its derivative.

[Claim 11] A method for introducing a physiologically active substance comprising a negative charge to cells, said method comprising a step of contacting the complex of Claim 9 or 10 with cells.

[Claim 12] A kit for preparing the composition of Claim 5, comprising phospholipid and a polyalkylenimine having two or more hydrophobic groups per molecule or its salt.

[Detailed Description of the Invention]

[0001]

[Technical Field of Industrial Application]

The present invention relates to compositions for transporting negatively charged substances into cells.

[0002]

[Prior Art]

In chemotherapy, a system for transporting a target drug into target cells or intracellular tissues, namely the Drug Delivery System (referred to as "DDS" hereafter), is an important technology. Needless to say, the introduction of a gene into

desired cells is a pivotal technology in gene therapy as well. There are two principal methods for introducing genes into cells.

[0003]

One is the method using a viral vector. This method includes a method for introducing a desired exogenous gene on the genome of a virus into cells by infecting with the virus. The other is the method in which a desired gene or a vector containing said gene is sealed-in or carried in an artificial or a semi-artificial carrier. This method comprises transporting substances into the desired organ (a target organ), cell (a target cell), or organelle (a target organelle) by letting each process relating to the behavior and transport of the subject *in vivo* depend on the physiochemical properties of the carrier itself. Carriers used in this method are, for example, liposomes (F. Ledley et al., Human Gene Therapy 6, 1129-1144, 1995), proteins (Human Gene Therapy 5, 429, 1994), peptides (Proceedings of National Academy of Sciences of United States of America 90, 893, 1993), synthetic macromolecular compounds (Tang et al., Human Gene Therapy 4, 823-832, 1997), and (reconstituted) Sendai viruses (Exp. Cell Res. 159, 399, 1985), etc.

[0004]

The method of using a liposome as a carrier has been contrived in many ways. Recently, based on the fact that a DNA molecule is a polyanion, the introduction of a DNA into a target cell was attempted by using, as a liposome, a polycationic lipid that has a static affinity to the DNA molecule, and which can easily form a complex. As such cationic lipids, lipofectin, 1,2-dioleoyloxy-3-(trimethylammonio)propane (DOTAP), 1,2-dimyristyloxypropyl-3-dimethylammonium bromide (DMRIE) (F. Ledley et al., Human Gene Therapy 6, 1129-1144, 1995), transfectum, etc., are known. However, there was a problem since cationic lipids are easily decomposed by lysosomes, and such, after been taken up into cells through the endocytosis process, and this lowers gene transfer efficiency. In addition, another problem was that cationic lipids show cytotoxicity when they are increased in order to improve introduction efficiency.

[0005]

To solve these problems, the use of cationic macromolecules has been attempted. For example, Kim et al. disclosed a gene introduction system in which polylysine is bound to a hydrophobic group, introduced into a plasma protein by hydrophobic interaction and statically bound to a DNA molecule (U.S. Patent No. 5,679,559). Zhou et al. prepared a liposome consisting of a polylysine into which a hydrophobic group was introduced, and a phospholipid (X. Zhou et al., Biochemica et Biophysica Acta. 1065, 8-14, 1991, X. Zhou et al., ibid. 1189, 195-203, 1994). Zhou et al. have successfully lowered cell damage by using poly-L-lysine and improved affinity of the DNA/liposome complex to a target cell by introducing lipid residues. However, in order to introduce the liposome into cells efficiently, the cells had to be processed with chemicals in advance. Also, WO97/45442 discloses a liposome including polyamine into which a cholesterol residue was introduced.

[0006]

As other cationic macromolecules, polyalkylenimines, particularly polyethylenimines (PEIs), are known (N. Oku et al., J. Biochemi. 100, 935-944, 1986, N. Oku et al., Biochemistry 26, 8145-8150, 1987, Suh et al., Bioorganic Chem. 22, 318-327, 1994). A polyethylenimine is a linear or branched polymer molecule comprising a protonated amino group in the molecule, through which a DNA molecule can be bound, and since no cell target substances or cell membrane denaturing agents have to be used, is applied for the introduction of genes (WO96/02655, O. Boussif et al., Proc. Natl. Acad, Sci. USA 92, 7297-7301, 1995, B. Abdallah et al., Hum. Gene Ther. 7, 1947-1954, 1996, Lambert et al., Mol. Cell Neurosci. 7, 239-246, 1996, R. Kircheis et al., Gene Ther. 4, 409-418, 1997, A. Baker et al., Gene Ther. 4, 773-782, 1997, A Baker et al., Nucleic Acids Res. 25, 1950-1956, 1997, Durmort et al., Gene Ther. 4, 808-814, 1997, Tang et al., Gene Ther. 4, 823-832, 1997, A. Boletta et al., Hum. Gene Ther. 8, 1243-1251, 1997, Ferrari et al., Gene Ther. 4, 1100-1106, 1997). Demeneix et al. have reported a hypothesis regarding the mechanism of introducing polyethylenimine into cells

(Artificial Self-Assembling Systems for Gene delivery, ed. by P. L. Felgner et al., p. 146-151, ACS conference proceeding series, 1996).

[0007]

However, a polyethylenimine to which a hydrophobic group has been introduced has not been reported as carriers for introducing genes.

[0008]

[Problems to Be Solved by the Invention]

An objective of the present invention is to provide a composition for the introduction of negatively charged substances into cells, which has a high gene-transfer efficiency, no toxicity towards cells, and a cationic macromolecule as the constitutive ingredient, and a method for introducing a negatively charged substance into cells using the composition.

[0009]

[Means to Solve the Problems]

To solve the above problems, the present inventors constructed a novel carrier, which comprises a polyethylenimine as a constituent, into which multiple hydrophobic groups have been introduced, and studied the introduction of genes into cells using the carrier. They found that a gene could be introduced into cells with a high transfer efficiency and extremely low toxicity by using this carrier.

[0010]

Specifically, the present inventors introduced multiple cetyl groups into a polyethylenimine to prepare the cetylated polyethylenimine shown in Figure 1, and prepared a carrier composition by mixing to the polyethylenimine, a phosphatidylethanolamine or a phosphatidylcholine, which are phospholipids. The obtained composition was mixed with a plasmid into which a fluorescent protein (GFP) was introduced, and the formed complex was introduced into COS-1 gene to examine the gene transfer efficiency by measuring fluorescent intensity and cell toxicity by measuring the cell density of living cells. As a result, the use of the carrier composition consisting of the

cetylated-polyethylenimine as a constitutive ingredient markedly increased the gene transfer efficiency and lowered extremely the toxicity against cells in comparison with the use of liposomes that have been conventionally used.

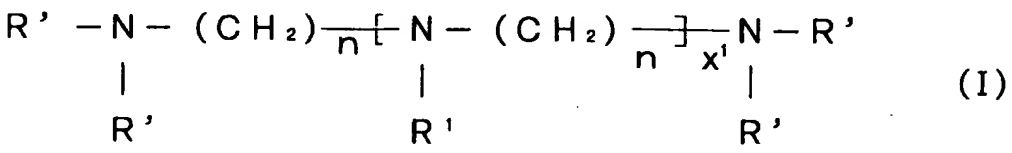
[0011]

Specifically, the present invention relates to a composition comprising polyalkylenimine into which multiple hydrophobic groups have been introduced, or its salt as an ingredient, and a method for introducing genes using the composition, and more specifically:

(1) a composition comprising polyalkylenimine having two or more hydrophobic groups, or its salt,

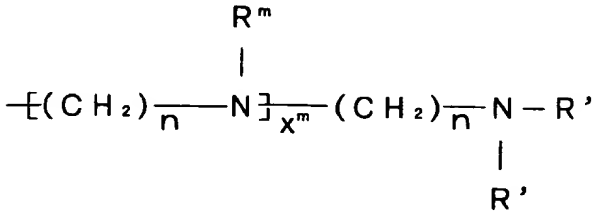
(2) the composition of (1), wherein the hydrophobic group is a cholesterol residue, a saturated or unsaturated alkyl group, a saturated or unsaturated acyl group, or a phospholipid residue,

(3) the composition of (1), wherein the polyalkylenimine having two or more hydrophobic groups is a compound represented by formula (I):

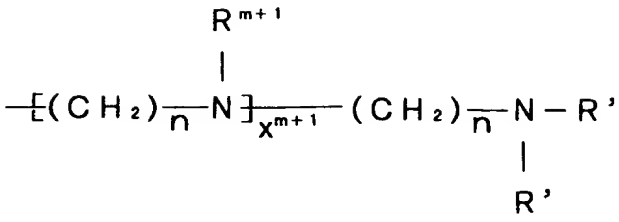


(I)

(wherein R' represents hydrogen, an alkyloxy group, or an alkoxy group; two R's binding to the same nitrogen atom can be identical or different; R^1 is hydrogen, a cholesterol residue, a saturated or unsaturated alkyl group, a saturated or unsaturated acyl group, a phospholipid residue, or below formula:



wherein R^m represents hydrogen, a cholesterol residue, a saturated or unsaturated alkyl group, a saturated or unsaturated acyl group, a phospholipid residue, or below formula:



wherein n, X^1, X^m, and X^{m+1} represent arbitrary natural numbers.)

(4) the composition of any one of (1) to (3), wherein the polyalkylenimine is a polyethylenimine.

(5) the composition of any one of (1) to (4), further comprising phospholipid,

(6) the composition of (5), wherein the phospholipid is a neutral or basic phospholipid,

(7) the composition of (5), wherein the phospholipid comprises phosphatidylethanolamine, or phosphatidylcholine skeleton,

(8) the composition of (7), wherein the phospholipid is dioleylphosphatidylethanolamine, or phosphatidylcholine,

(9) a complex comprising a physiologically active substance comprising a negative charge and a composition of any one of (1) to (8),

(10) the complex of (10), in which the physiologically active substance comprising a negative charge is a nucleic acid or a derivative thereof,

(11) a method for introducing a physiologically active substance comprising a negative charge to cells, said method comprising a step of contacting the complex of (9) or (10) with cells,

(12) a kit for preparing the composition of (5), comprising phospholipid and polyalkylenimine having two or more hydrophobic groups per molecule or its salt.

[0012]

[Mode for Carrying out the Invention]

The present invention relates to a composition comprising polyalkylenimine in which two or more hydrophobic groups have been introduced per molecule, or its salt. In the present invention, "polyalkylenimine" is meant by a polymer molecule comprising a

protonated amino group, and may be a linear or branched. An alkylenmonomer, a constitutive unit of polyalkylenimine, is preferably a lower alkylenimine comprising 1 to 10 carbon atoms, and more preferably a lower alkylenimine with 1 to 3 carbon atoms from the viewpoint of water-solubility. From the viewpoint of convenience of synthesis, particularly, polyethylenimine is preferable. A polyethylenimine can be prepared by the methods known to one skilled in the art (for example, refer to Examined Published Japanese Patent Application (JP-B) No. Sho 43-8828, U.S. Patent No. 4,032,480, and U.S. Patent No. 4,467,115]. Alternatively, commercial products [for example, polyethylenimine (molecular weight 600), product name EPOMINE (Nippon Shokubai), product name ExGen 500 (Euromedex), etc.], may be used. The average molecular weight among polyethylenimines used in the present invention is usually 200 to 1,000,000, and preferably 300 to 500,000, and more preferably 500 to 100,000.

[0013]

Any hydrophobic group that improves affinity between polyalkylenimine and phospholipid can be used as a hydrophobic group that is introduced into polyalkylenimine. For example, a cholesterol residue, a saturated or unsaturated alkyl group, or a saturated or unsaturated acyl group, or a phospholipid residue can be used. Preferably a cetyl group, a stearyl group, and an oleyl group can be used. To enhance the transfer efficiency of negatively charged substance into cells, at least two hydrophobic groups are introduced into a polyalkylenimine molecule. Introduction of too many hydrophobic groups lowers the water-solubility of polyalkylenimine, therefore is not preferable. One skilled in the art can conveniently choose the appropriate number of hydrophobic groups in a molecule. The hydrophobic group introduced into polyalkylenimine may bind to polyalkylenimine through a spacer. A neutral water-soluble molecule is preferable as a spacer. For example, an amino acid, a peptide, a polyamino acid, a protein, a sugar, a synthetic macromolecule, such as a polyethylene glycol, a polyvinyl alcohol, a polyvinyl pyrrolidone, a dextran derivative, etc., and their derivatives can be used.

In the case of using as a carrier for transporting negatively charged substances, polyalkylenimine may form a salt. As a salt of polyalkylenimine, for example, hydrochloride, sulfate, phosphate, carbonate, formate, oxalate, citrate, succinate, and such, can be used, but is not limited thereto.

[0014]

The polyalkylenimine prepared in this manner into which multiple hydrophobic groups are introduced, can be used as a carrier to transport negatively charged substances by itself. Moreover, by mixing with a phospholipid to form a liposome, it can be used as a carrier. The polyalkylenimine into which the hydrophobic group was introduced presumably exists stably in the liposome as the hydrophobic group comprises a feature of being stable inside of the phospholipid. As a phospholipid used for the formation of the liposome, neutral or acidic lipid that does not interact with a physiologically active substance comprising negative charge by itself is preferable. Phospholipids may derive from nature or be synthetic. As an alkyl side chain of the phospholipid used in the present invention, one with 12 to 18 carbon atoms, or an oleyl group are preferable. As a phospholipid, for example, dioleoylphosphatidylethanolamine comprising the phosphatidylethanolamine skeleton, or phosphatidylcholine comprising the phosphatidylcholine skeleton (for example, derived from egg-yolk, soybean, or synthetic one), are suitably used. Any mixing ratio of phospholipid and polyethylenimine can be used as long as the mixture is positively charged in the range of comprising electrical affinity to a negatively charged substance. One skilled in the art can conveniently choose the preferable ratio of mixing.

[0015]

The present invention also relates to a complex comprising a physiologically active substance with a negative charge and the above composition. Any physiologically active substance with multiple negative charges can be used as the physiologically active substance with a negative charge. For example, a nucleic acid and its derivative can be used. As a nucleic acid, circular or linear, single or double stranded deoxyribonucleic acid, or

ribonucleic acid can be used. As a derivative of nucleic acids, for example, phosphorothioate, phosphorodithioate, etc., can be given. In the complex of the present invention, the mixing ratio of the above composition against the physiologically active substance comprising a negative charge is preferably 1/10 equivalent to 20 equivalents at the charge ratio. One skilled in the art can conveniently choose an appropriate mixing ratio. The particle size of the complex is preferably 200 nm or less, and more preferably 100 nm or less. In the preparation of the complex of the present invention, another adjuvant is not necessary, however, as an adjuvant, amphipathic molecules, etc., such as phosphatidylethanolamine or phosphatidylcholine, fatty acid, etc., can be used.

[0016]

In the case of using polyalkylenimine of the present invention in the transport of a negatively charged substance by itself, a complex is formed by directly mixing the negatively charged substance, and the negatively charged substance can be introduced into cells by following known methods (O. Boussif et al., Proc Natl. Acad. Sci. USA 92, 7297-7301, 1995). While in the case of forming a liposome by mixing polyalkylenimine of the present invention with a phospholipid, polyalkylenimine of the present invention is mixed with a phospholipid, such as dioleylphosphatidylethanolamine (DOPE, Nippon Seika), or phosphatidylcholine (eggPC, Nippon Seika) to prepare an aqueous dispersion of the liposome by known methods (N. Oku et al., Biochim. Biophys. Acta, 1280, 149-154, 1996). In this case, the liposome may contain a sterol group, such as cholesterol, as a membrane stabilizer, and tocopherol, vitamin E, etc. as an antioxidant. In the case of lipidmicrosphere, a target lipidmicrosphere can be obtained by mixing polyalkylenimine of the present invention, soybean oil, and detergent by means of known methods (F. Liu et al., Pharmaceutical Res. 13, 1642-1646, 1996). A negatively charged substance can be introduced into cells by mixing a lipidmicrosphere with negatively charged substances, and contacting with cells.

[0017]

The present invention also relates to a kit for preparing liposome, comprising phospholipid and polyalkylenimine in which two or more hydrophobic group have been introduced per molecule or its salt. The kit of the present invention can contain the above membrane stabilizer, and antioxidant as well as phospholipid and a polyalkylenimine having two or more hydrophobic per molecule or its salt. The final product of the phospholipid sample and the polyalkylenimine sample contained in the kit of the present invention can be cooled, frozen, or lyophilized. In the case of the lyophilized product, sorbitol, sucrose, amino acid and various proteins, etc., can be contained as a stabilizer.

[0018]

[Examples]

The present invention is illustrated in detail by Examples, but is not to be construed as being restricted thereto.

[0019]

[Example 1] Purification of polyethylenimine

Commercially available polyethylenimine (molecular weight 600, product name: EPOMINE SP-006, Nippon Shokubai) (2 g) was dissolved in 100 ml of distilled water and ultrafiltered through a ultrafiltration membrane of a molecular cut off of 500 (UH-05, Toyo Paper Filter, the ultrafiltration device was manufactured by Amicon, stirring type cell model 8,400), under 2 to 3 kg/cm^2 nitrogen stream, with 1000 ml of distilled water. The ultrafiltrate in which impurities were removed (about 20 to 30 ml) was lyophilized to obtain purified polyethylenimine.

[0020]

[Example 2] Synthesis of cetylated polyethylenimine

In 20 ml of chloroform, 1 g of purified polyethylenimine (molecular weight: 600) and 1.62 g of cetyl bromide were mixed (molar ratio, 1:2), 1 ml of triethylamine was added thereto and refluxed. Subsequently, unreacted polyethylenimine was removed using an ultrafiltraion membrane with a molecular cut off of 1000 (YM1, Amicon). Yield of lyophilized cetylated polyethylenimine was 70.4%. The number of the introduced cetyl groups on cetylated

polyethylenimine was confirmed by NMR. Specifically, the proton ratio of cetyl group at around 1.3 ppm and polyethylenimine at around 2.5 to 3.5 ppm supported that 3 molecules of cetyl groups bound to one molecule of polyethylenimine. Figure 1 shows the synthesized cetylated polyethylenimine.

[0021]

[Example 3] Preparation of liposome

To prepare a liposome, a chloroform solution of 10 mM cetylated polyethylenimine and a chloroform solution of 10 mM dioleylphosphatidylethanolamine (DOPE, Nippon Seika) were prepared beforehand. The lipid composition used was cetylated polyethylenimine/DOPE = 0.65/1 (molar ratio). This lipid was dissolved in chloroform in a pear-shaped flask, chloroform was removed under reduced pressure by a rotary evaporator, and a lipid film was prepared. This lipid film was dried under high vacuum for one hour, hydrated with DMEM (Gibco BRL) and adjusted to 1 mM. Freeze-thawing was repeated three times and the liposome was treated by ultrasonication for 10 min in a bath-type ultrasonicator. Even though liposomes are stable, they were however, prepared when necessary.

[0022]

As a comparative example, 1,2-dimyristyloxypropyl-3-dimethylhydroxyethylammonium bromide (DMRIE), or 1,2-dioleyloxy-3-(trimethylammonio)propane (DOTAP) was used in stead of cetylated polyethylenimine to obtain liposomes in the same manner as in the above.

[0023]

[Example 4] Preparation of plasmid DNA/liposome complex

pEGFP-C1 (Clontech) was used as the plasmid DNA. This plasmid encodes GFP (green fluorescent protein) as a reporter gene to quantify the gene expression by measuring fluorescent intensity. The plasmid was prepared by using *E. coli*, purified by the cesium chloride density-gradient centrifugation method, dissolved in TE buffer (pH 7.5) to 2μg/ml. The quantity of plasmid DNA was kept constant, transferred to a 1.5-ml Eppendorf tube and 1 mM liposome solutions with various ratios were added thereto. DMEM was added

to adjust the volume to 100 µl and incubated at room temperature for 20 min.

[0024]

[Example 5] Introduction of genes

COS-1 cells were seeded in a 35-mm dish to 1 x 10^5 cells per dish, and cultured at 37°C in the presence of 5% CO_2 overnight. The cells were washed with DMEM twice. The prepared liposome/plasmid DNA complex was diluted with serum-free DMEM and added to cells to adjust the total volume to 250 µl. The cells were incubated at 37°C in the presence of 5% CO_2 for three hours. After 3 hours, the liposome/plasmid DNA complex solution was removed, washed with serum-free DMEM twice, 2 ml of 10% FBS-DMEM was added thereto, and the cells were incubated for 48 hours.

[0025]

[Example 6] Quantification of the expression of the introduced gene (GFP)

The medium was removed from the above cells incubated for 48 hours. The cells were washed with serum-free DMEM twice. Triton X-100 solution was added thereto to a final concentration of 1%, and the cells were incubated at room temperature for 30 min for solubilization. The cell suspension was transferred to an Eppendorf tube and centrifuged at 3,000 rpm for 10 min. The amount of gene expression was determined by measuring fluorescent intensity at Ex 493 nm, and Em 510 nm. Figure 2 shows the result. When the composition of the present invention was used, the expression was twice or higher in comparison with the controls of DMRIE and DOTAP.

[0026]

[Example 7] Gene expression in the case of using the comparative liposome

The expression of the introduced gene was measured in the same manner as in Examples 1 to 6, except for using egg-yolk-derived phosphatidylcholine (eggPC) instead of DOPE in Example 3. Figure 3 shows the result. In the case of using eggPC, the gene expression was similar to that in DOPE.

[0027]

[Example 8] Examination of cytotoxicity

One milliliter of COS-1 cells were added to each well of a 24-well plate (Corning), and incubated at 37°C in the presence of 5% CO_2 overnight until confluency. Each well was washed with DMEM twice, and 200 µl of the prepared plasmid DNA/liposome complex solution of various concentrations was added into each well, incubated at 37°C in the presence of 5% CO_2 for three hours. In the same manner, DMEM-only and liposome solution-only were examined as controls.

[0028]

[Example 9] Measurement of cell density

Cytotoxicity was measured by using AlamerBlue (Biosource International, imported by Iwaki Glass). Each sample solution was removed and 200 µl of serum-free DMEM and 50 µl of AlamerBlue diluted into 1/5 were added to each well. After 1 hour of incubation at 37°C in the presence of 5% CO_2, the solution in each well was transferred to a 1.5-ml Eppendorf tube, and 750 µl of PBS(-) was added thereto to make a total volume of 1 ml. Fluorescent intensity was measured at Ex 535 nm and Em 583 nm. Figures 4, 5, and 6 show the results. In the case of using the composition of the present invention, the cytotoxicity was extremely lowered, especially at the high concentration of the complex solution, compared with using the conventional liposome.

[0029]

[Effects of the Invention]

The present invention provides a composition comprising as an ingredient, a polyalkylenimine into which multiple hydrophobic groups have been introduced, and a method for introducing genes using said composition. Thus, the present invention enables a more effective transport of negatively charged physiologically active substances to cells with low cytotoxicity when compared to using conventional cationic macromolecules.

[Brief Description of the Drawings]

[Figure 1]

Structure of polyethylenimine used in Examples into which hydrophobic groups had been introduced.

[Figure 2]

GFP gene expression when the charge ratio of a plasmid and a liposome was varied in the case of using the composition of the present invention, and a conventional liposome. In the figure, PCL indicates tricetyl-PEI: DOPE (0.65:1, molar ratio; molecular weight of polyethylenimine, 600). Similarly, DMRIE:DOPE (1:1, molar ratio), and DOTAP: DOPE (1:1, molar ratio) are shown, and COS-1 cell was used. The error-bar over the plot indicates ±SD.

[Figure 3]

The effect of phospholipid on gene expression. In the figure, DOPE-PCL indicates tricetyl-PEI:DOPE (0.65:1, molar ratio), eggPC-PCL indicates tricetyl-PEI: eggPC (0.65:1, molar ratio).

[Figure 4]

The cytotoxicity when the concentration of the liposome of the present invention alone or of the plasmid complex was changed. In the figure, PCL indicates tricetyl-PEI:DOPE (0.65:1, molar ratio; molecular weight of polyethylenimine, 600). COS-1 cell was used. The error bar indicates the plus side of ±SD.

[Figure 5]

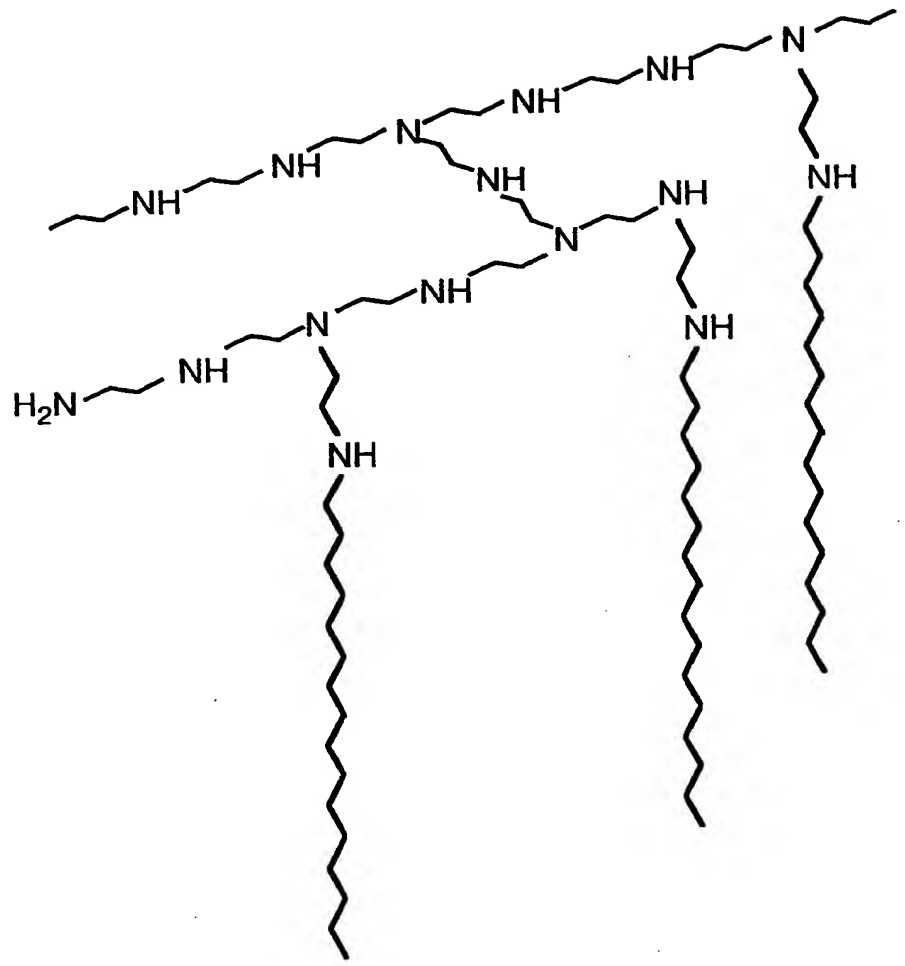
The cytotoxicity when the concentration of the conventional liposome (DMRIE) alone or of the plasmid complex was changed. In the figure, DMRIS lipo. indicates DMRIE: DOPE (1:1, molar ratio). COS-1 cell was used. The error-bar indicates the plus side of ±SD.

[Figure 6]

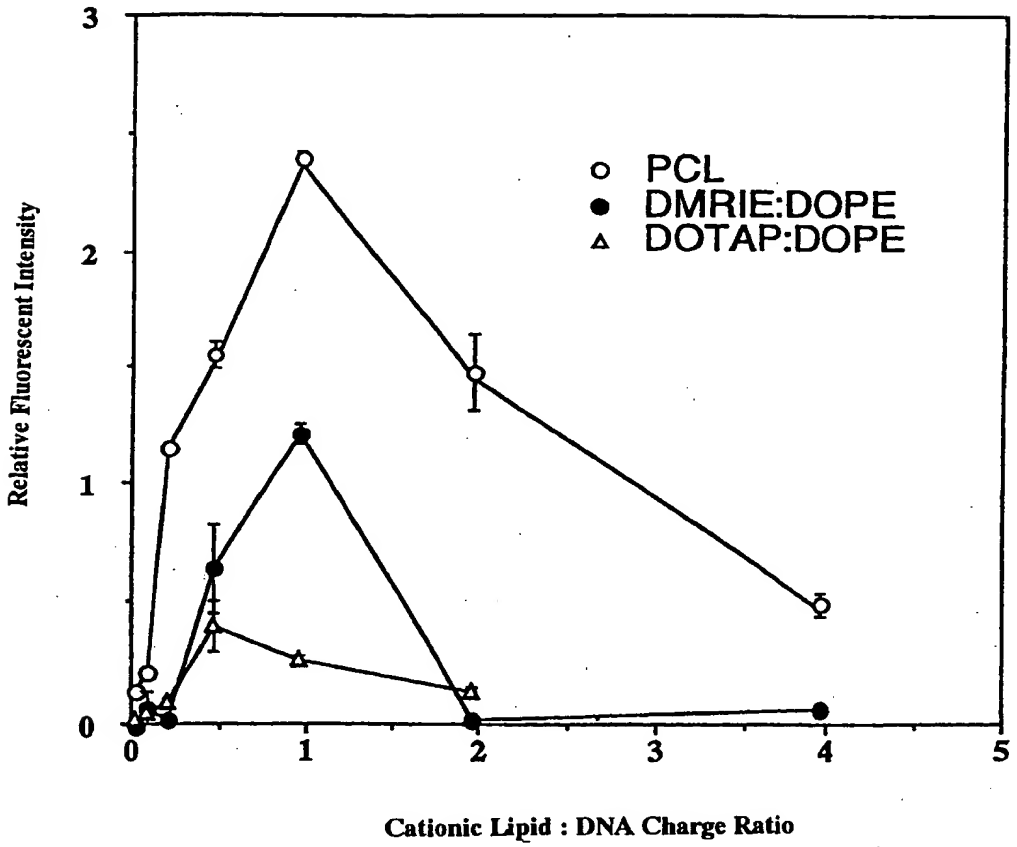
The cytotoxicity when the concentration of the conventional liposome (DOTAP) alone or of the plasmid complex was changed. In the figure, DOTAP lipo. indicates DOTAP:DOPE (1:1, molar ratio). COS-1 cell was used. The error-bar indicates the plus side of ±SD.

[Document Name] Drawings

[Figure 1]

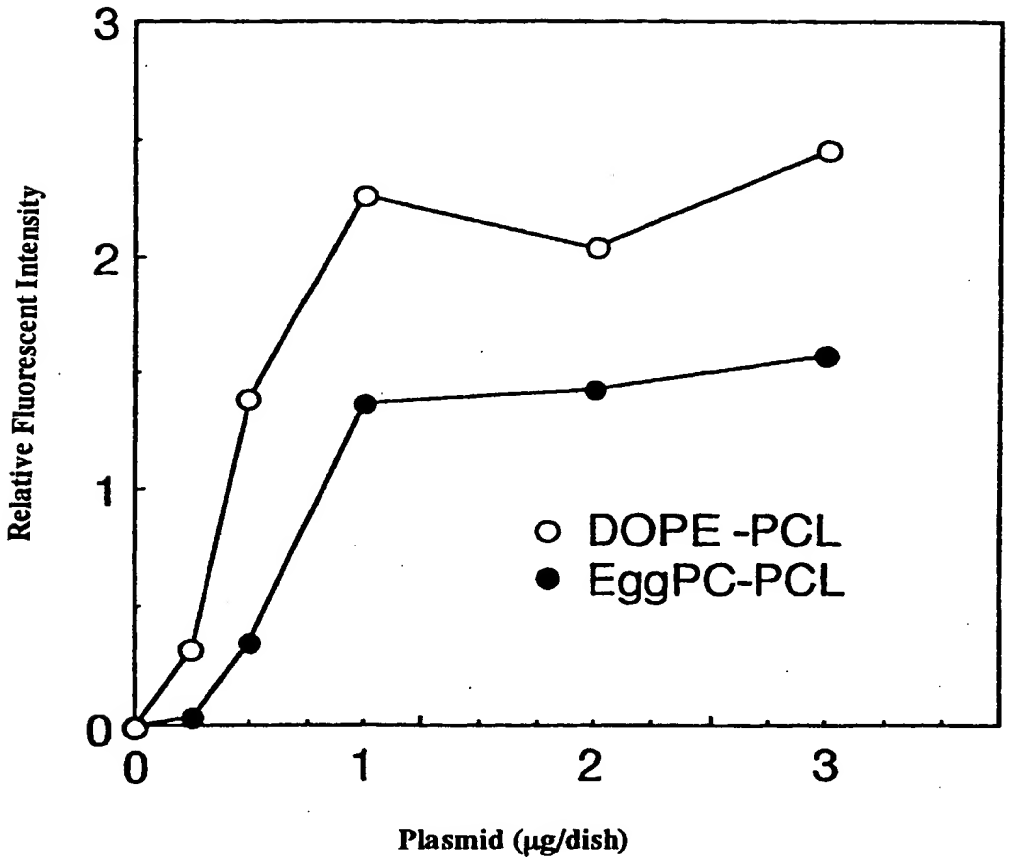


[Figure 2]

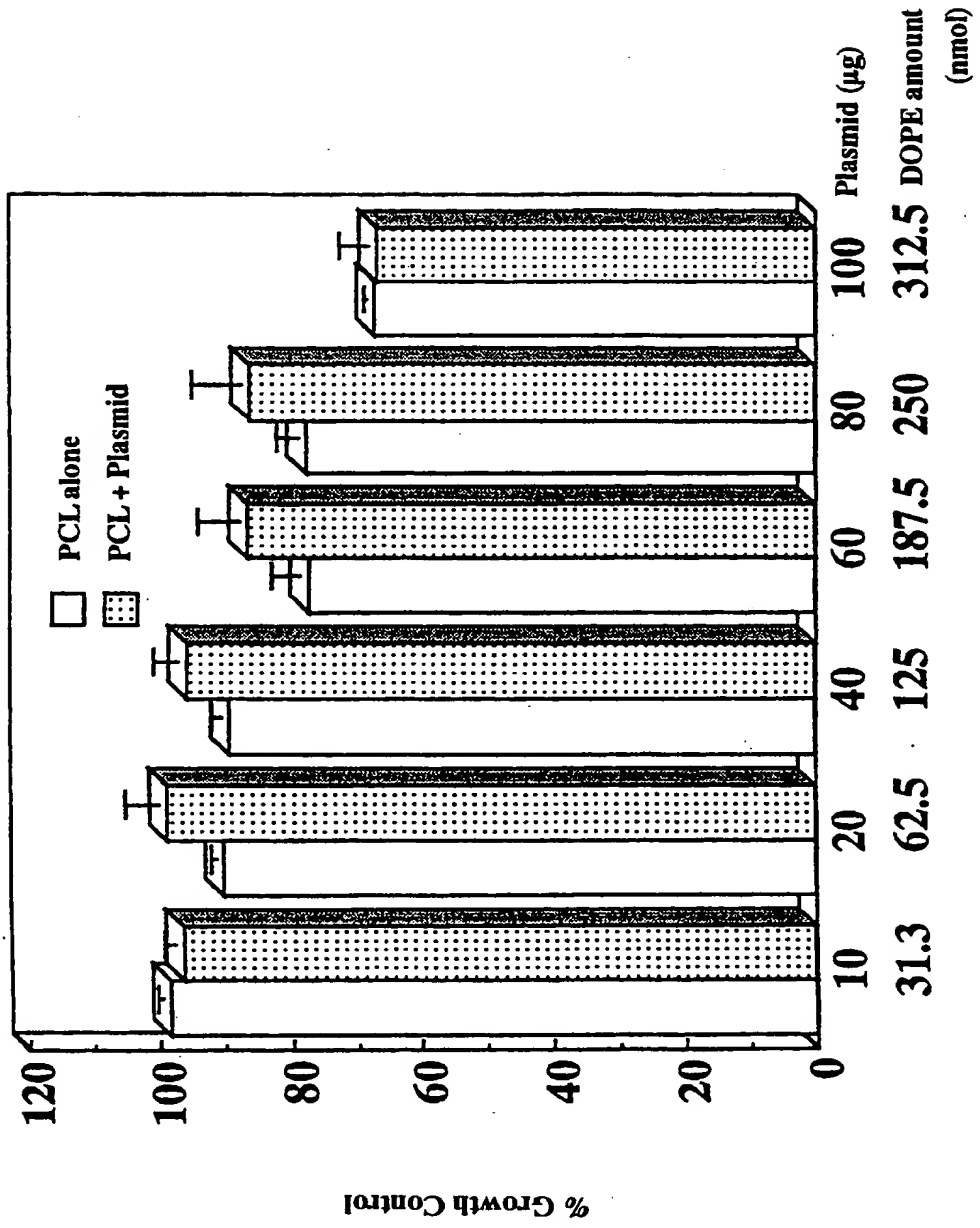

3
2
1
0
0
1
2
3
4
5
Relative Fluorescent Intensity
Cationic Lipid : DNA Charge Ratio
PCL
DMRIE:DOPE
DOTAP:DOPE

5

[Figure 3]

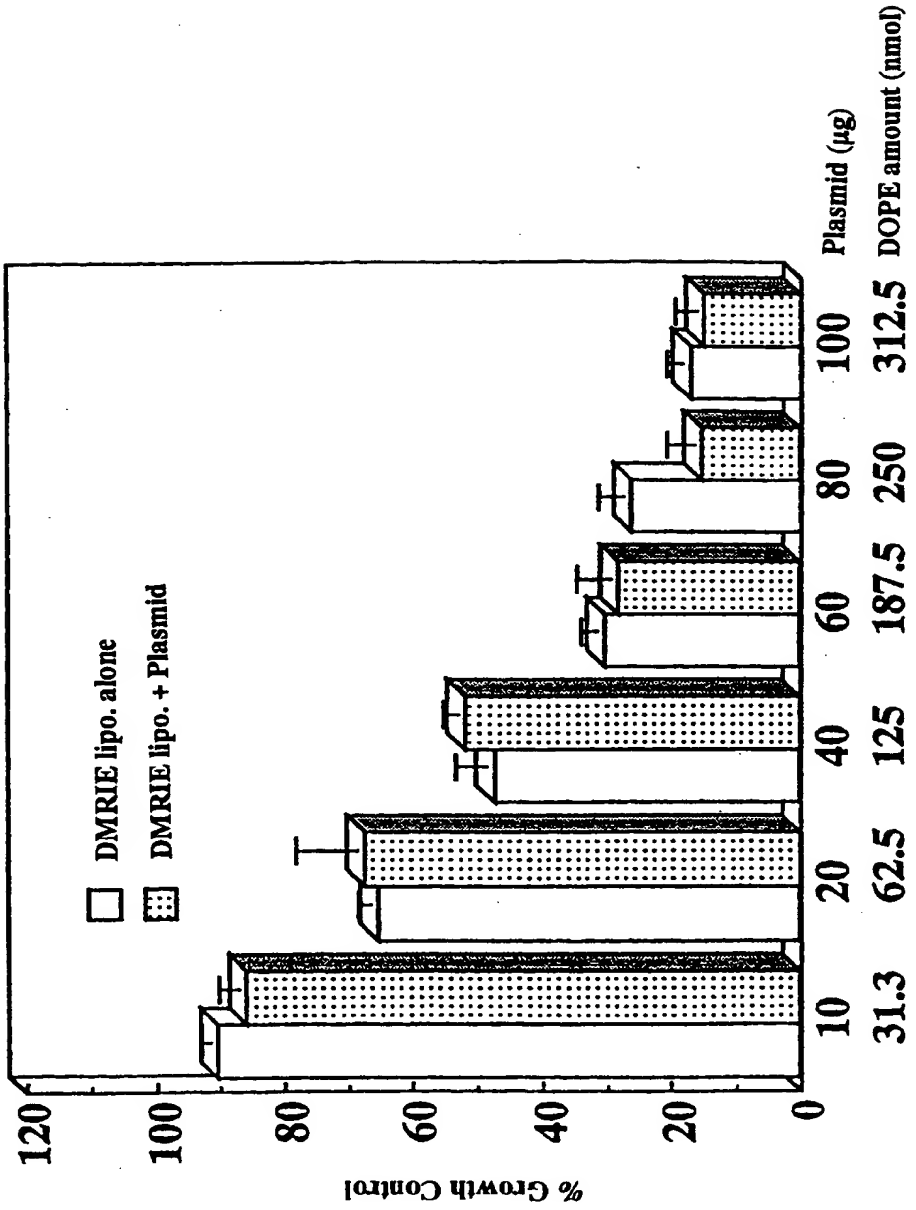

Relative Fluorescent Intensity
Plasmid (μg/dish)
DOPE -PCL
EggPC-PCL
0
1
2
3

[Figure 4]

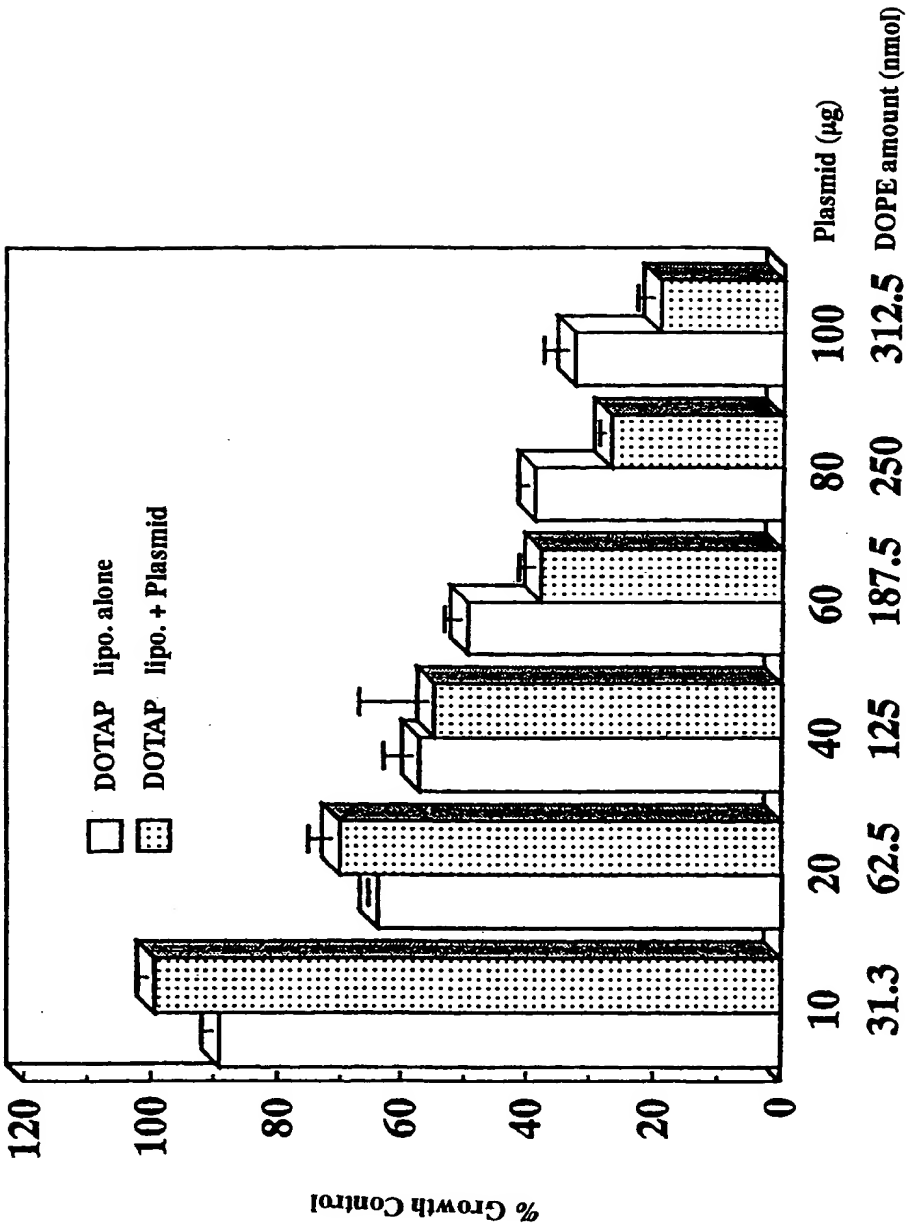

% Growth Control
120
100
80
60
40
20
0
PCL alone
PCL + Plasmid
10
20
40
60
80
100
Plasmid (μg)
31.3
62.5
125
187.5
250
312.5
DOPE amount
(nmol)

5

[Figure 5]


% Growth Control
120
100
80
60
40
20
0
DMRIE lipo. alone
DMRIE lipo. + Plasmid
10
20
40
60
80
100
Plasmid (µg)
31.3
62.5
125
187.5
250
312.5
DOPE amount (nmol)

[Figure 6]


% Growth Control
120
100
80
60
40
20
0
DOTAP lipo. alone
DOTAP lipo. + Plasmid
10
20
40
60
80
100
Plasmid (μg)
31.3
62.5
125
187.5
250
312.5
DOPE amount (nmol)

[Document Name] Abstract

[Abstract]

[Problems to Be Solved] An objective of the present invention is to provide a composition for the introduction of negatively charged substances into cells, which has a high gene-transfer efficiency, no toxicity towards cells, and a cationic macromolecule as the constitutive ingredient, and a method for gene transfer using the composition.

[Means to Solve the Problems] Novel transport carriers comprising polyalkylimines having two or more hydrophobic groups. These carriers enable the transfer of genes into cells at a high transfer efficiency.

[Selected Drawing] None